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Light Reception: Discovering the Clock-Eye in Mammals

Dispatch

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Light is the most reliable environmental signal for adjusting biological clocks to the 24-hour day. Mammals receive this signal exclusively through the eyes, but not just via rods and cones. New evidence has been uncovered for a novel photoreceptor that may be responsible for more than just adjusting the clock.

Even without time cues from the environment, physiological events, from gene expression to behaviour, recur with a high regularity but not necessarily in a precise 24 hour rhythm — hence the term ‘circa-dian’ (‘about one day’). Circadian rhythms are controlled by endogenous ‘clocks’ which are synchronised, or ‘entrained’, to the 24-hour day predominantly by light [1]. The central circadian pacemaker in mammals resides above the optic chiasm in the suprachiasmatic nuclei (SCN). It has long been known that light entrainment in mammals requires the eyes, but it was unclear through which photoreceptor the signal was processed. It came as a surprise that the circadian clock remains perfectly entrainable by light in mutant mice devoid of rods and cones [2].

Scientists are racing to identify the novel receptor in the mammalian retina. Its spectral characteristics have been defined in mice and, more recently, in humans [3,4]. In addition to its role in entrainment, the novel photoreceptor is responsible for several other, non-visual light responses, such as melatonin suppression, pupillary constriction and direct effects of light on motor-activity (‘masking’) (see references in [5]), or for many other ‘vegetative’ light effects, for example on cortisol levels [6] or heart rate [7]. Results reported recently in *Current Biology* [5] take our understanding of this novel light input pathway a step further, showing that its influence is already apparent in the primary steps of intra-retinal signal processing.

Vision and Irradiance Detection: Different Systems for Different Tasks

Vision capitalises on photons, using rods or cones as ‘pixels’ to create a retinal image that is processed in the thalamus and the cortex. While a memory of these ‘pictures’ may be stored in the brain, the retinal picture itself has to be renewable within milliseconds for instant detection of any changes. Visual processing thus requires both fast kinetics and high spatial resolution.

In contrast, a detector for the assessment of day and night should not care about a flash of lightning or the shadow of a flying object. Its task is to integrate photons over a long time. This integration mechanism is partially responsible for the difficulties that shift workers have in adjusting their biological clocks to socially enforced schedules — the competition between indoor and outdoor light cannot be won. A worker who is exposed to 500 lux over an eight-hour night shift collects a similar quantity of photons waiting 15 minutes for the bus, even on a cloudy day. As a result, the circadian system remains entrained to the ‘real’ day — it cannot adjust to the implemented night shift, so workers try to be active and alert when their physiology is tuned to sleep. In fact, workers on night shifts, with most of the rest of the day free to spend outdoors, may collect more day light than their non-shifting colleagues. The invention of artificial light has ironically created a biological shadow world because we spend more time indoors.

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Spectral Action

What is the basis for irradiance detection without rods or cones? Opsins are not the only photoreceptors. In *Drosophila*, circadian light reception can use cryptochrome [8], a flavo-protein with similarities to light-dependent DNA repair enzymes. Several light receptors collaborate in resetting the circadian clock of plants, including cryptochromes and phytochromes [9]. Cryptochromes are also present in mammals and are, in fact, tightly linked to the molecular circadian machinery, although their role as mammalian photoreceptors is disputed [10,11].

Most of the recent insights into circadian light reception pathways are based on gene mutation and ablation studies. This approach can generally only be used to associate a gene product with a light-dependent process. It is only when genetic analyses are combined with action spectra that a photopigment function can be implied. In this approach, the strength of an appropriate light response — such as shifting the circadian clock or suppression of melatonin — is measured for many different fluences and wavelengths, and the results are converted to an action spectrum that indicates the receptor’s absorption characteristics.

The first action spectra in rodless and coneless mice, using pupillary constriction as readout, indicated an opsin-type photoreceptor with a peak absorption around 479 nm [12], different from all the classical opsins in the mammalian retina. The action-spectrum approach was subsequently applied to suppression of melatonin by light in humans [3,4], and again, the results indicate a novel photoreceptor with absorption maxima at 464 and 459 nm, respectively.

The most recent action spectrum constructed for humans [5] used yet another readout, namely primary intra-retinal signal processing, and offers a basis for some predictions made over a decade ago [13]. Hankins *et al.* [14] had found that the ‘on’ and ‘off’

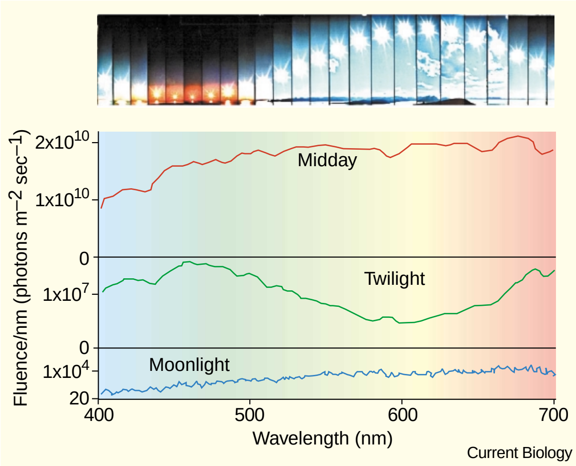

Figure 1. Spectral changes during the natural day.

The top panel shows a series of photographs taken throughout a mid-summers day north of the artic circle. Note that the light is enriched in yellow-red photons when the sun's rays have to travel further through the atmosphere at the horizon. The lower panel shows the spectral composition of light coming from above at midday, dawn/dusk and during a moon-lit night (redrawn after [20]). In contrast to the twilight received from the horizon, that from above is relatively poor in yellow-red photons. In addition to irradiance, the light input responsible for detecting night and day and other vegetative light responses could, therefore, use colour as information. The spectral differences of twilight between the horizon and the sky's dome would also create a gradient on the retina, with light from above falling primarily onto the lower retinal hemisphere.

responses of cones, as measured by the electroretinogram (ERG), depend on prior light history. When a brief flash of light, in the milliseconds range, is presented under controlled adapted conditions, the ERG response consists of a series of systematic downs and ups. The first trough and peak represent the cones' response to the onset of the stimulus, while later ones correspond to the off response. When the retina has been dark adapted for some time, the appearance of the first ERG peak is significantly later than if the retina had previously seen light. The influence of a 15 minute light exposure on this ERG latency is still seen after several hours. This sensitive and highly reproducible effect has now been used to perform the labour intensive task of generating an action spectrum [5], by testing monochromatic light for its effectiveness in long-term adaptation. The results indicate that the light signal is transduced by an opsin-based photoreceptor with a peak absorption around 483 nm.

The Colours of Time

The opsin-like absorption maxima derived for melatonin suppression, on the one hand, and for intraretinal processing, on the other, are 20 nm apart. Future experiments will show whether this difference actually indicates two distinct, novel opsins. The fact that different photoreceptors have distinct spectral characteristics does not necessarily mean that the system can 'see' colours. Colour reception requires that at least two, spectrally different receptors interact in their signal processing. In view of the fact that light environments are noisy — clouds pass, animals burrow, and so on — it would be advantageous if the circadian clock could exploit spectral qualities in addition to irradiance.

Many factors that vary light intensity are spectrally neutral, while the transitions of dawn and dusk are accompanied by significant spectral changes (Figure 1). If the light-entrainment pathway could use colour as information, in addition to irradiance, it could even compare different areas of the visual field. At twilight, for example, the horizon is enriched in the yellow-red part of the spectrum (Figure 1, top), whereas the light from above, which falls predominantly on the lower half of the retina, is poorer in these wavelengths (middle part of Figure 1, bottom).

The fact that circadian clocks have the ability to use spectral information for entrainment has been known since the late eighties [15]. In the unicellular marine organism *Gonyaulax*, red and blue light have opposing effects on the circadian clock, and the multiple light inputs in higher plants also have differential effects on phase and period [9]. An involvement of multiple light inputs is also indicated in mammals: data emerging from Russell Foster's group suggest that the loss of rods and cones in mutant mice changes the spectral response for phase shifting the clock, and indicate that both classical and novel photoreceptors play a part in light entrainment (R. Foster, personal communication).

Most laboratories use simple, rectangular on–off transitions in their light:dark regimes. Yet, several studies show that these artificial conditions obscure other important qualities of the natural light transitions. European ground squirrels, for example, never experience dawn or dusk at any time of year, because they come up from their burrows after sunrise and go back before nightfall [16]. Because they can still adjust to seasons, it is thought that their circadian clocks track the daily irradiance changes which vary throughout the year. Others have shown that slow irradiance changes, as in nature, entrain differently and even more efficiently compared to rectangular light changes [17]. To distinguish signal from noise, the circadian system and its inputs may have to use as many parameters as possible in addition to irradiance itself, such as the colour and the direction of the *zeitgeber* light.

A View to the Future

The intelligent experimental approach by Hankins and Lucas [5] forms an excellent basis for finding the relevant light parameters that circadian clocks use for entrainment. If, for example, the direction of the light source plays some role in assessing time of day, one would expect the irradiance detectors to be unevenly distributed over the upper and the lower part of the retina. The controlled design of these experiments allows detailed retinotopic measurements in subjects to investigate which parts of the retina contribute primarily to the underlying long-term adaptation process, and whether adaptation can be transferred from one part of the retina to another, similar to information transfer between the two eyes.

Basic knowledge about this novel light input pathway will open up many possibilities for understanding and treating circadian-clock-related syndromes in industrialised society. Compared to a farmer spending all day outdoors, the circadian clock of an office worker receives the daily light changes with a greatly reduced amplitude. Perhaps this is why more and more sighted people are being diagnosed with sleep disorders, such as extremely early or late sleep onsets [18,19]. These syndromes could reflect the extremes of a genetic heterogeneity that was not apparent under strong *zeitgeber* conditions. Detailed knowledge about the spectral characteristics of the non-image-forming novel light input(s) may even help shift workers to win the competition between indoor and outdoor light, for example, by excluding certain photons of the natural light with the help of spectacles when they spend time outside.

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